

# Reducing Chilling Injury and Maintaining Quality of Horticultural Crops with Natural Products and Their Derivatives

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## Abstract

Most tropical and subtropical crops are susceptible to chilling injury. They can not take the advantage of refrigeration to lengthen their storage and shelf lives because of the development of chilling injury symptoms when exposed to low temperatures. Therefore, how to reduce chilling injury and maintain quality of these crops after harvest becomes an important task. We have found that certain natural products and their derivatives seem to be effective in delaying the onset and reducing the severity of chilling injury symptoms. Of many natural products that we have tested, methyl jasmonate (MJ) and methyl salicylate (MS) were the most beneficial. These two natural volatile compounds consistently reduced chilling injury in mangoes, papayas, peppers, tomatoes, and zucchini squashes. MJ and MS were also found to enhance the resistance of tissues to chilling injury by increasing the gene expression of heat shock proteins, pathogenesis-related proteins, and alternative oxidase. Recent results also showed that MJ increased antioxidant capacities, antioxidant enzyme activities, and free radical scavenging capacities in the tissues. These results indicate that MJ can act to prevent chilling injury by a mechanism which involves protecting tissues from free radical injury.

## INTRODUCTION

The development of recommendations for the beneficial use of naturally occurring or GRAS (generally recognized as safe) substances and sustainable non-chemical techniques to minimize our dependence on potentially hazardous chemicals is needed to reduce chilling injury and postharvest losses of fruits and vegetables.

Several naturally occurring or GRAS compounds have shown promise in reducing postharvest diseases and disorders in horticultural crops. Methyl jasmonate (MJ), either as a vapor or as an emulsion, has been shown to reduce microbial contamination of fresh-cut celery and peppers (Buta and Moline, 1998), inhibit gray mold infection in strawberries (Moline et al., 1997), suppress green mold growth in grapefruit (Droby et al., 1999), and control Botrytis rot in cut rose flowers (Meir et al., 1998). Methyl jasmonate also decreased the severity of postharvest brown rot in sweet cherries when used as a co-fumigant with thymol or carvacrol (Tsao and Zhou, 2000). In addition, methyl jasmonate delays decay and pitting development caused by chilling injury in zucchini squash (Wang and Buta, 1994), avocados, grapefruit, peppers (Meir et al., 1996), mangoes (Gonzalez-Aguilar et al., 2001), and peaches (Feng et al., 2003). Ethanol (EtOH) has also been found to have antimicrobial properties, and a postharvest ethanol dip eliminated most of the fungal and bacterial populations on the surface of grapes without impairing bunch appearance or berry firmness (Lichter et al., 2002). Ethanol vapor also prevented scald development in apples (Ghahramani et al., 2000; Chervin et al., 2001) and reduced leaf blackening in the stems of cut flower *Protea* 'Pink Ice' (Crick and McConchie, 1999). Allyl isothiocyanate (AITC) has been shown to have strong antimicrobial activity in both liquid and vapor forms (Delaquis and Sholberg, 1997; Isshiki et al., 1992; Lin et al., 2000). AITC is a constituent of cruciferous vegetables and is produced during the

enzymatic degradation of glucosinolate. The bactericidal activity of AITC against pathogens on iceberg lettuce and tomatoes has also been demonstrated (Lin et al., 2000). The essential oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO), is becoming increasingly popular, particularly as a naturally occurring antimicrobial agent (Carson and Riley, 1995). Tea tree oil was effective in inhibiting the storage pathogen *Botrytis cinerea* on Dutch white cabbage (*Brassica oleracea* var. Capitata) (Bishop and Reagan, 1998). Its vapor phase has shown a high level of antifungal activity against 15 common postharvest pathogens on a variety of crops (Bishop and Thornton, 1997). Acetic acid (AA) or vinegar vapor was effective in preventing conidia of brown rot, gray mold, and blue mold from germinating and causing decay of stone fruit, strawberries, and apples (Sholberg et al., 2000). Acetic acid vapor also reduced postharvest brown rot of apricot and plums (Liu et al., 2002). Exposure of avocado fruit to acetaldehyde vapor delayed fruit softening and inhibited ethylene production (Pesis et al., 1998). Because these natural products have been shown to be successful in maintaining quality of these crops, there is a high potential for successfully incorporating them into an integrated strategy with peppers, tomatoes, squash, and other chilling-sensitive produce. The physiological changes in these commodities affected by the natural volatile treatments and the mechanisms by which these natural products exert their effect will be discussed.

## MATERIALS AND METHODS

Several naturally occurring substances, particularly those that can be used as fumigants, were used to treat intact and fresh-cut peppers, squash, tomatoes, mangoes, papayas, and other chilling-sensitive produce. Concentrations of natural volatile compounds evaluated were: methyl jasmonate, 0.01-0.1 mM; methyl salicylate, 0.1 mM; allyl isothiocyanate, 2-5 ml/L; absolute ethyl alcohol, 200 ml/L; essential oil of *Melaleuca alternifolia*, 100 ml/L; and acetic acid, 4 ml/L. The optimal treatment strategy that maintains quality and includes method of treatment, dose (treatment concentration and duration), and temperature were determined for each commodity. The mechanism(s) by which these natural products exerted their effect were investigated. A molecular biological approach was used to examine the expression of genes affected by methyl jasmonate, methyl salicylate, and other naturally occurring substances. Specific defense-related genes investigated include: class I and II small heat shock proteins and 70 KD high molecular weight heat shock protein family; pathogenesis-related proteins (e.g. pr-2a, 2b, 3a and 3b); and alternative oxidase genes (e.g. 1.5 and 3.5 kb). Criteria for selection and specific targets were tolerance to low temperature injury, resistance to decay, and maintenance of texture and flavor. Total RNA were extracted and northern analysis were conducted to determine the effect of these natural products on the expression of those genes according to methods described previously (Ding et al., 2001, 2002; Fung et al., 2004).

The effect of natural products on nutritional value, specifically antioxidant content including flavonoid, anthocyanin, and phenolic compounds were analyzed. Retention of the antioxidant capacity (measured as oxygen radical absorbance capacity) of the treated samples were compared to those of the untreated commodities. The activities of various antioxidant enzymes including superoxide dismutase (SOD), guaiacol peroxidase (G-POD), ascorbate peroxidase (AsA-POD), glutathione peroxidase (GSH-POD), glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) were analyzed according to methods outlined by Chanjirakul et al. (2006).

## RESULTS AND DISCUSSION

### Effect of Natural Products on Chilling Injury and Quality of Fruits and Vegetables

Different natural products have different degrees of effectiveness in reducing chilling injury and maintaining quality of fruits and vegetables. MJ and AITC were the most effective, followed by TTO, EtOH, and AA in retarding decay in raspberries (Table

1) (Wang, 2003; Chanjirakul et al., 2006). MJ was also more effective than isopropyl alcohol, 1-propanol, or methyl alcohol in maintaining the quality of fresh-cut kiwifruit (Wang and Buta, 2003). The beneficial effect of MJ in alleviating chilling injury has also been demonstrated in mangoes, papayas, guavas, and zucchini squash (Gonzalez-Aguilar et al., 2001, 2003 and 2004; Wang and Buta, 1994).

### Effect of Natural Products on Gene Expression in Chilling-Sensitive Crops

The expression of several defensive genes in chilling-sensitive species has been reported to be enhanced by treatment with MJ or MS. These genes include heat shock proteins (Fig. 1) and pathogenesis-related proteins in tomatoes (Ding et al., 2001; 2002), and alternative oxidase in sweet peppers (Fung et al., 2004). The increased transcript abundance of these proteins was correlated with protection against chilling injury. These results indicate that MJ or MS treatment could increase the resistance to chilling injury and pathogen invasion in chilling-sensitive crops by elevating the expression of these genes.

### Effect of Natural Products on Antioxidant Activity in Horticultural Crops

Many natural products which showed beneficial effect in reducing chilling injury and decay also were found to increase the antioxidant activity and free radical scavenging capacity in fruit tissues (Fig. 2). The activities of antioxidant enzymes such as superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, glutathione peroxidase, glutathione reductase, monodehydroascorbate reductase, and dehydroascorbate reductase were promoted by most of the natural volatile compounds tested (Chanjirakul et al., 2006), indicating that high antioxidant enzyme activities may help in alleviating oxidative stress and in turn increase the resistance of tissues against chilling injury and decay. One notable exception is AITC treatment. While AITC showed good results for decay inhibition in most commodities, it did not increase the antioxidant capacity or the antioxidant enzyme activities. Thus, AITC may retard the decay directly by its antimicrobial properties or by a different mechanism.

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Table 1. Effect of various volatile compounds on the severity of decay in raspberry fruit after 10 days of storage at 10°C.

Volatile compound	Concentration ( $\mu\text{L}\cdot\text{L}^{-1}$ )	Score <sup>a</sup>
Methyl jasmonate	22.4	1.0 $\pm$ 0.0 <sup>b</sup>
Allyl isothiocyanate	5.0	1.0 $\pm$ 0.0
Tea tree oil	100.0	1.7 $\pm$ 0.3
Absolute ethyl alcohol	200.0	1.2 $\pm$ 0.3
Acetic acid	4000.0	3.0 $\pm$ 0.0
Control	--	3.3 $\pm$ 0.5

<sup>a</sup>Scoring represents a visual rating of decay severity on raspberry fruit using a scale of 1 to 5 with 1 = no infection, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe infection.

<sup>b</sup>Mean value  $\pm$  S.E.

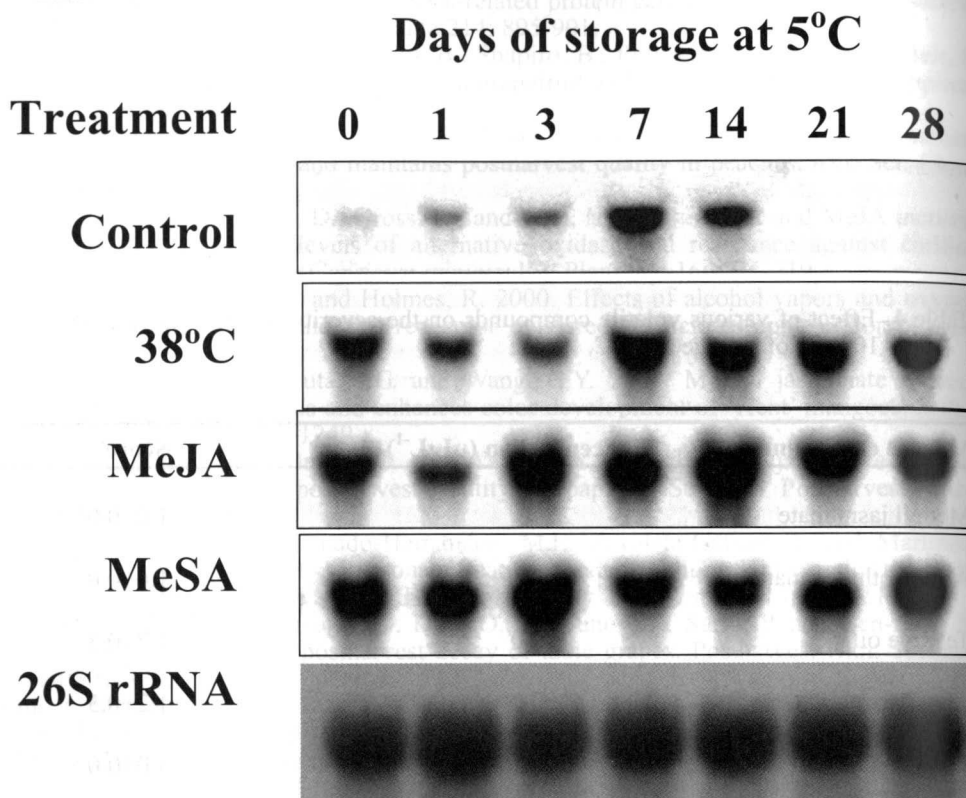


Fig. 1. Expression of heat shock protein (HSP 17.6) gene transcripts in different pretreated tomato fruit during chilling temperature storage.

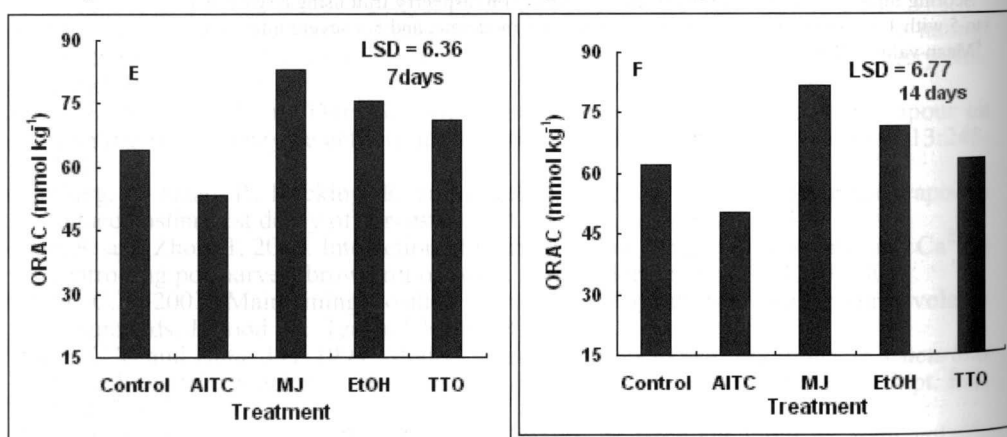


Fig. 2. Antioxidant activity (measured as oxygen radical absorbance capacity, ORAC) in raspberry fruit treated with various natural products and stored for 7 and 14 days at 10°C.